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THE STUDY OF THE MICROMORPHOLOGY, ULTRA-STRUCTURE AND PHYSIOLOGY OF INSECT CENTRAL NERVOUS SYSTEM

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13. ABSTRACT

Research on glutamic acid decarboxylase in the gaba shunt in the supraosephageal ganglion of the honey bee was completed and was published in the Journal of Insect Physiology. This work is of significance as it relates to the possible identity of an additional mediator in the neural junction of the central nervous system. A number of experiments have been carried out during the past year on retinular cells, axons associated with retinular cells, and those tissues associated with the visual sensory receptors. This study continues to be important in determining the relationship of neural fibers associated with the compound eye and should continue to be helpful in explaining how certain lower animals accomplish visual discrimination and orientation. In the studies of the external and internal surface of the compound eye of the fleshfly, Sarcophaga bullata it was noted that there is a low pattern corneal nipple and sparse sitiform; also inter-facetal hairs have been observed on the corneal lens surface. Particular cleavage planes revealed outlines of the Semper cells, their nuclei and distant terminations of photoreceptor cells. These have been described with their axonal processes. These axons have been noted as traversing the external chiasma and entering the lamina gaglionaris where suggestions of synaptic contact have been pointed out. These descriptions with the scanning electron microscope have been correlated with previous work on the transmission electron microscope.

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During the past year the following research has been completed on the Air Force Grant for the continuation of The study of the micromorphology, ultrastructure and physiology of insect central nervous system. Recent studies made on neuromusculature and nerve-nerve relationships and the possible mediation of neural transmitter substances have essentially been completed and this research has been set aside for the present time. Dr. Michael Fox has completed his research on glutamic acid decarboxylase in the gaba shunt in the supraosephageal ganglion of the honey bee, Apis mellifera. This work has appeared in the Journal of Insect Physiology and a reprint of this work is attached to this report. We feel that this work has been of great significance inasmuch as that there are few people carrying on research in this particular area of neurochemistry at this time. Dr. Fox has accomplished an excellent piece of research on the possible identity of an additional mediator in the neural junction of the central nervous system. A determination of the chemical identity of synaptic transmitter agents is a major problem in neurophysiology. Inasmuch as a great majority of central nervous system synapses and neuromuscular junctions involve chemical transmission, an adequate study and a full understanding of these structures will be possible only when the transmitter agents have been characterized. We feel the work carried out by Dr. Fox is significant knowledge to the overall picture of neurotransmitter agents.

With the additional purchase of freeze-dry equipment, purchased on this grant, and the availability of the scanning electron microscope a number of experiments have been carried out during the past year on retinular cells,

axons associated with retinular cells, and those tissues associated with the visual sensory receptors. This study continues to be important in determining the relationship of neural fibers associated with the compound eye and should continue to be helpful in explaining how certain lower animals accomplish visual discrimination and orientation.

In conjunction with Dr. Stanley Carlson who has been at this University on a postdoctoral position a great deal of work was started using the scanning electron microscope in association with the Pearce freeze-dry apparatus. Out of this work has come the publication of two papers in the German journal Zeitchtschrift fur Zellforschung und Mikroskopische Anatomie. Thus far we have studied the external and internal surface of the compound eye of the fleshfly, Sarcophaga bullata. We have noted that there is a low pattern corneal nipple and sparse sitiform also inter-facetal hairs have been observed on the corneal lens surface. Particular cleavage planes revealed outlines of the Semper cells, their nuclei and distant terminations of photoreceptor cells. These have been described with their axonal processes. These axons have been noted as traversing the external chiasma and entering the lamina ganglionaris where suggestions of synaptic contact have been pointed out. These descriptions with the scanning electron microscope have been correlated with previous work on the transmission electron microscope. We have also carried out some studies on the superposition eye in the sphingid moth, Manduca sexta. We have specifically examined the corneal nipples, the corneal lens, crystalline cones and tracts, photoreceptor cells and their axons. Description of the external ultrastructure of the components were correlated where possible with previously published accounts of internal ultrastructure as obtained from

transmission electron microscope studies. One of the key findings in this work was the demonstration of the axial rotation of the eccentrically situated retinular cell, its externally noted prominence and the arrangement of the other photoreceptor cells composing the retinulum. Because of the interest in superposition eye theory the functional significance of various pre-retinular optic components was reviewed where it specifically related to Manduca. These two papers represent a brief beginning and an indication of what can be accomplished using the instrumentation available in this laboratory. It is of course our intent to continue an extensive study of the photoreceptors using this technique.

Also completed during the past year on AFOSR-71-2065 was some work on the sensory receptors located on the ligula of the mosquito Culiseta inornata. This work now has been set aside and more concentrated effort is being put on the visual receptors. However, some rather fascinating work was completed identifying the lack of sensory receptors on the ligula and pointing out more exact relationship in the mouth parts of the mosquito. This work was published with Dr. William Owen at the University of Wyoming and has appeared in the Transactions of the American Microscopical Society.

Studies currently being carried out in this laboratory which were started on AFOSR-71-2065 are of the ultrastructure of the dorsal eye of the water beetle Dineutes assimilis. This is a gyrinid beetle which is unique in that it lives at the interface and possesses two dorsal compound eyes situated above the water line and two ventral compound eyes situated below the water line. Studies on the ultrastructure and mechanism of light and dark adaptation are currently being carried out.

The ultrastructure of the dorsal eye has been explored with the transmission electron microscope. Sufficient cross sectional material has been obtained to begin reconstruction of the receptors. Epon embedded material sectioned with a diamond knife at 1.8 microns and photographed with the light microscope is being used to supplement the transmission electron microscope sections.

Transmission electron microscopy of the ventral eye is underway, again supplemented with light microscopy. The ventral receptors are arranged in a three-tiered rhabdom similar to those of the dorsal eye. Ultrastructural differences from the dorsal receptors are apparent in the material examined though these must be confirmed with examination of additional ventral eyes. The amount of pigment surrounding the ventral cones appears greater than in the dorsal eye. Multivesicular bodies are a prominent intracellular component in the dorsal receptors where only a few onion bodies are found. The frequency of finding these two intracellular organelles is reversed in the ventral eye. In dorsal and ventral eyes the intermediate rhabdom area is formed by six rhabdomeres and has the shape of a St. Andrew's cross. Cross sections of this area in the ventral eye differ in two respects from the dorsal receptors. In the dorsal receptor the microvilli of the six cells are lined up parallel to one another in one of two perpendicular directions. This plan is repeated in the ventral eye in general but the microvilli are not as straight and follow a rather wavy course. Also of interest are the large number of small vesicles at the base of ventral microvilli of the intermediate rhabdom. These vesicles appear as infoldings of the cell membrane between adjacent microvilli and are not present in the dorsal eye. These differences in ultrastructure are of

interest with relation to the greater ultraviolet sensitivity of the ventral eye (Bennett 1965, 1967).

Scanning electron microscopy was done on specimens fractured parallel to the length of the retinular cells. This exposed for view the receptor layer from lens to basal membrane and will be of some use in the receptor reconstruction.

Material is now in preparation for studying light and dark adaptation in dorsal and ventral eyes. The light adapted specimens are fixed and embedded according to the same procedure used for transmission electron microscope specimens. Dark adapted specimens are fixed briefly with boiling water and then treated the same as light adapted specimens. The epon blocks are sectioned at 1.8 microns with a diamond knife. Subsequently the epon is removed from the sections and they are stained with toluidine blue. Light and dark adapted dorsal eye sections have been photographed and preliminary assessment shows a crystalline tract of some 10 to 15 microns in length in the light adapted state. In the dark adapted condition the distal rhabdomere appears to extend right up to the base of the crystalline cone and the crystalline tract is not seen. Ventral eye material is still in preparation. This material has been viewed with phase contrast though phase photographs have not yet been made.

Bennett, R.R. 1965. Vision in the whirligig beetle Dineutes ciliatus Forsbg. Tufts University, thesis.

Bennett, R.R. 1967. Spectral sensitivity studies on the whirligig beetle, Dineutes ciliatus. Journal of Insect Physiology 13:621-633.